

## THE MATING SEQUENCE OF THE BENTHIC ARROWWORM *SPADELLA SCHIZOPTERA*

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### ABSTRACT

The mating behavior of the hermaphroditic arrowworm, *Spadella schizoptera*, was followed photographically. The mating behavior consists of four distinct actions: vertical swinging of the worm's body, touching each other with their heads, standing erect face-to-face, and sperm transfer. During sperm transfer, one worm (donor) jumps suddenly, ejaculates a sperm cluster, and transfers it to the partner (recipient). The well-ordered mating sequence leads to accurate placement and transfer of the sperm to the genital orifice of the partner. We concluded that sperm transfer in *Spadella schizoptera* is non-reciprocal: one worm acts as a male and the other as a female.

### INTRODUCTION

The chaetognaths, or arrowworms, are mostly planktonic and, in general, are not easily maintained in the laboratory. Behavioral aspects of their reproduction such as mating and egg-laying have been relatively ignored, compared to our understanding of the maturation process of sperm and eggs (Stevens, 1910; Burfield, 1927; John, 1933; Jägersten, 1940; Ghirardelli, 1968; Alvarino, 1983a, b).

The arrowworms are hermaphroditic; some species are capable of both cross- and self-fertilizations (*cf.* Reeve and Cosper, 1975). For the planktonic species, *Sagitta hispida*, Reeve and Walter (1972) have shown photographs of worms just before and after sperm transfer. The mating behavior of the benthic species, *Spadella cephaloptera*, has been studied by Vasiljev (1925), John (1933), and Ghirardelli (1953, 1968). According to Vasiljev (1925) and Ghirardelli (1953, 1968), sperm transfer is reciprocal, each worm acts simultaneously as a male as well as a female. On the other hand, John (1933) has described it as non-reciprocal: one worm acts only as a male and the other, only as a female.

While breeding *Spadella schizoptera* in the laboratory, we found that mating behavior is different from that reported for *S. cephaloptera*. We describe an ordered sequence of mating that leads to sperm transfer in *S. schizoptera*.

### MATERIALS AND METHODS

Specimens of *Spadella schizoptera* were collected in March of 1983 and 1984 from tide pools near the Amakusa Marine Biological Laboratory, Kumamoto Pref. The temperature of the seawater was about 14°C. All animals were taken to the Ushimado Marine Laboratory and kept in seawater-filled vessels (25 cm diameter, 10 cm depth) in a room maintained at a constant temperature (14°C) having a cycle of 12 h light and 12 h darkness. Ten worms were placed in each vessel. Seawater was changed every three days, at which time food (either *Artemia nauplii* or *Tigriopus*) was added. Only mature worms were used for behavioral observations. In these worms, the seminal

vesicles were completely filled with sperm, although the ovaries contained varying sized ova.

Mating behavior was induced when more than three worms in 50 ml seawater were transferred to room temperature (approximately 18–20°C). Successful sperm transfer was observed more than a hundred times. In order to follow the sequence of mating behavior, 10 mature worms were placed in a square vessel (8 cm × 8 cm, 3 cm depth) and photographed from the side at 2–5 s intervals. The camera system enabled single as well as multi-exposures using strobe flashes.

Transferred sperm clusters were observed using a Hitachi 610 scanning electron microscope. For this purpose, we fixed worms which had not mated for several hours, worms which had just received a sperm cluster, and worms which were laying eggs. All specimens were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer containing 0.4 M sucrose (pH 7.4) and rinsed in the same buffer for 2 h each. After dehydration through a graded series of ethanol followed by substitution with isoamyl acetate, the worms were dried in a critical point drying apparatus and coated with gold.

## RESULTS

Unless disturbed, *Spadella schizoptera* stays on a substrate, usually attached by its adhesive fins. Sometimes they detach and dart a short distance. When the worm population density and the seawater temperature were raised, the worms became active swimmers, reattaching to the substrate occasionally. In a resting state, some of those worms began to swing their bodies vertically 2–5 times, while remaining attached to the substrate using the adhesive fins as a fulcrum (Fig. 1A, right worm). In general, these movements lasted approximately 1–2 s, and recurred several times with only a few seconds interruption. When two worms came within a distance of about 5 mm, they often started a mating sequence as shown in Figure 1a–f. In such cases, at least one of the worms exhibited the swinging movement. The second worm, which had not swung its body previously, started a swinging movement in response. The initial swinging movements thus appear to be a kind of signaling behavior. The two worms then moved nearer to each other (Fig. 1b).

The two worms eventually contacted one another with their heads (Fig. 1c), then moved closer and suddenly stood erect, assuming a face-to-face (ventral to ventral) position (Fig. 1d). After about 5 to 30 s, one sprang up momentarily and hit its partner with its tail (Fig. 1e). Immediately after the hit, a sperm cluster appeared on the side of the worm that was hit (Fig. 1f, the arrow on the left worm), while the other worm exhibited seminal vesicles which appeared largely exhausted as evidenced by changes in color and size. The hitting action appears to transfer the sperm; we will refer to the worm that hits the partner as a donor and the other one as a recipient. After sperm transfer, the two worms swam apart. The whole sequence of mating behavior varied in duration, from less than 10 s to a few min. On rare occasions, the same couple would immediately remate by reversing their roles as donor and recipient.

The transfer of a sperm cluster is the most important event in the mating sequence. The worm has a pair of seminal vesicles and it is possible for the donor to transfer two clusters to the recipient during a single mating. Figure 2 shows a successful case of a simultaneous double transfer. The transfer process was too rapid to be photographed. Sperm were released as a cluster from the apical region of the seminal vesicle in response to touching the seminal vesicle gently with a needle (Fig. 3a, b). Sperm cluster attachment always occurred on the ventral side of the body, just anterior to the lateral fins (see Fig. 2). SEM observations of egg-laying worms revealed a large pore (70 μm diameter, Fig. 4) which corresponds to the region where the sperm cluster

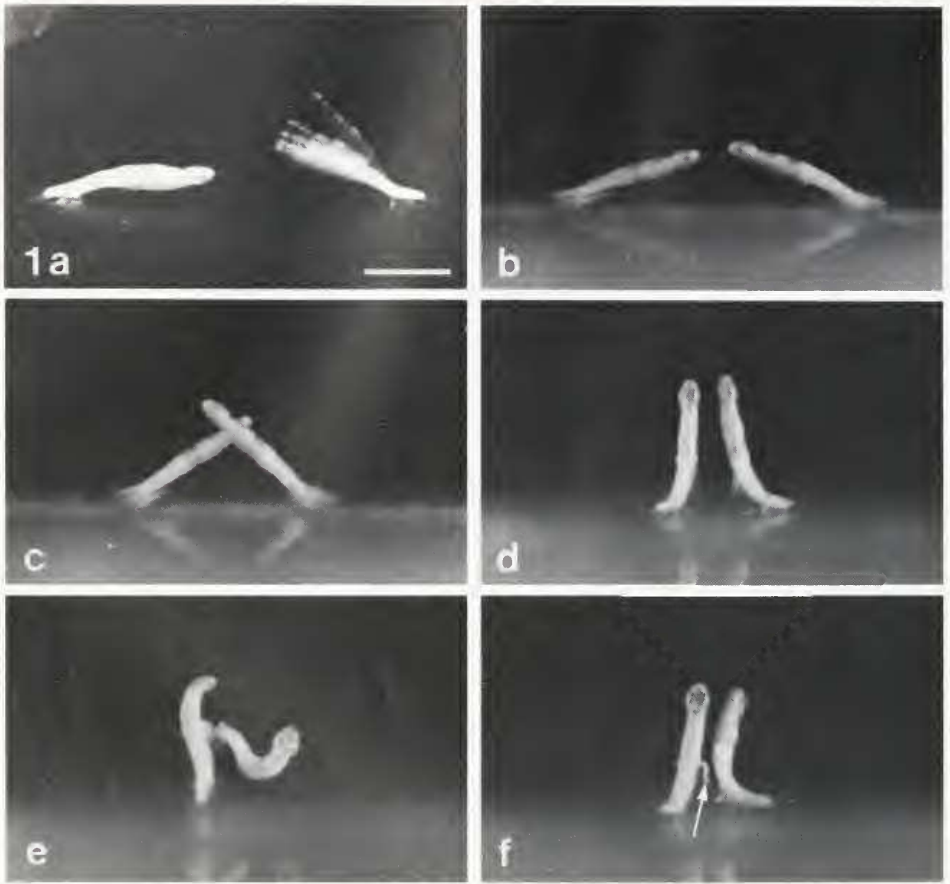


FIGURE 1. Sequence of mating behavior of *Spadella schizoptera*. (a) Two worms approach, the right worm has initiated swinging movements. (b) The two worms move nearer to each other. (c) They eventually touch each other with their heads. (d) Suddenly they become erect, assuming a face-to-face position. (e) The right worm hits its partner with its tail. (f) The left worm can be seen with a sperm cluster (arrow) on its side. The time required from encounter (a) to copulation (e) was from less than 10 s to a few min. The pictures, (a), (b, c), and (d, e, f) were taken with different couples. Scale bar: 2 mm.

was to be attached (arrows in Fig. 5a). The pore appears to be enlarged at the time of egg-laying. The observed pore is apparently a genital orifice through which sperm enter (Fig. 5b) and eggs exit.

#### DISCUSSION

*Spadella schizoptera* can be maintained in the laboratory for several months. Mating behavior is frequently observed when the worms are placed in conditions of high density and elevated temperature. According to Ghirardelli (1968) the mating behavior of *S. cephaloptera* occurs mostly in the late afternoon. In its natural environment, *S. schizoptera* is found in small populations of about 10 to 20 worms per 900 cm<sup>2</sup> in the lower intertidal zone. Such population densities are not high, but apparently are adequate for conspecific contacts. Moreover, the temperature of tide pools rises during

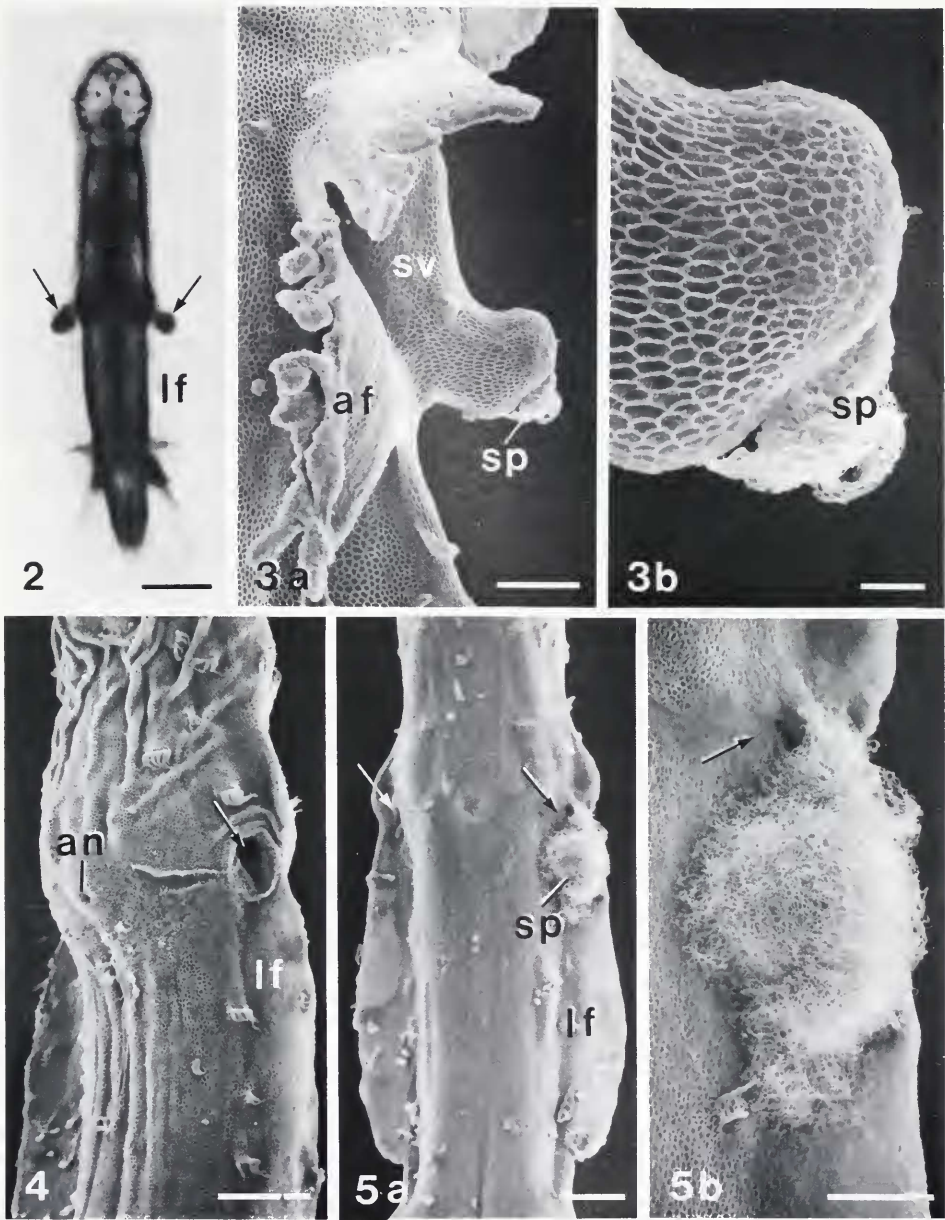


FIGURE 2. Dorsal view of a worm that received a pair of sperm clusters (arrows) which were attached to the ventral side, a little anterior to the lateral fins (lf). Scale bar: 500  $\mu$ m.

FIGURE 3. (a) SEM of the seminal vesicle (sv) observed from the ventral side. At higher magnification (b), the sperm are just extruded from the apical region of a seminal vesicle which was touched with a needle. af, adhesive fin; sp, sperm. Scale bars: a = 50  $\mu$ m; b = 10  $\mu$ m.

FIGURE 4. SEM of the ventro-lateral region, a little anterior to the lateral fin of an egg-laying worm. The genital orifice (arrow) is clearly seen. an, anus; lf, lateral fin. Scale bar: 100  $\mu$ m.

FIGURE 5. (a) SEM of the ventral region of a worm which has just received sperm (sp). The sperm which were pushed out as a cluster are attached near one of the genital orifices (arrows). (b) Higher magnification of the genital orifice (arrow) through which sperm appear to be entering. lf, lateral fin. Scale bars: a = 100  $\mu$ m; b = 50  $\mu$ m.

the day. This daytime condition resembles the conditions which induce mating behavior in the laboratory.

During mating of *S. schizoptera*, four actions occur sequentially: vertical body swinging, head touching, body alignment, and sperm transfer. The swinging movements (Fig. 1a, right worm) are repeated several times until sperm transfer is induced. These movements may be a key stimulus for the initiation of the mating sequence. What kind of conditions, internal or external, are needed for the worms to swing and how does one partner recognize its swinging counterpart? Photo- (*cf.* Goto and Yoshida, 1984) and mechano-senses (Horridge and Boulton, 1967; Fraser, 1969; Feigenbaum and Reeve, 1977) have been described, both behaviorally and morphologically. Since the simple pigment-cup eye of chaetognatha does not form an image, a vibration sense appears to be a candidate for partner recognition. The ranges of the vibration sense which has been revealed using the feeding response of starved worms as criteria are from 9 to 20 Hz in *S. cephaloptera* (Horridge and Boulton, 1967) and from 8 to 140 Hz in *S. schizoptera* (Feigenbaum and Reeve, 1977). Careful observations of satiated worms under low frequency vibration is needed.

When two worms encounter each other, they touch one another with their heads (Fig. 1c) as if they were trying to estimate the distance between themselves. Similar behavior has been described in a closely related species, *S. cephaloptera* (John, 1933; Ghirardelli, 1968); however, the body alignment (Fig. 1d) followed by the unusual copulation observed in *S. schizoptera* (Fig. 1e) has not been reported.

Cannibalism is common in this species. Interestingly, the worms that begin to swing are never cannibalized; they concentrate on mating.

According to Vasiljev (1925) and Ghirardelli (1953, 1968), mating specimens of *S. cephaloptera* align in parallel, taking a head-to-tail position and simultaneous sperm transfer occurs. Vasiljev (1925) states that sperm are directly placed on the genital orifice whereas Ghirardelli (1953, 1968) observed that sperm are first placed on the neck region and later move toward the genital orifice. In *S. schizoptera*, paired sperm clusters (Fig. 2) are transferred non-reciprocally from one animal to a point near the genital orifice of another animal (Figs. 4 and 5a). The transferred sperm appear to move through the genital orifice (arrow in Fig. 5b) toward the seminal receptacles.

These observations suggest that for successful sperm transfer, three requirements have to be fulfilled: (1) the face-to-face alignment of partners, (2) the partners must be the appropriate distance from each other, and (3) the proper orientation during jumping and hitting must be achieved. Improper placement of sperm clusters was occasionally observed, due perhaps to the failure of completing one of the above steps.

Ejaculation may be induced by the mechanical stimuli of the hitting action at the time of sperm transfer. This is supported by the fact that light touching with a needle results in ejaculation (Fig. 3a, b). As described by Alvariño (1983b), the released sperm are transferred as a cluster (Fig. 5a, b) not in the form of a spermatophore as is true in arthropods and gastropods. Reeve and Coper (1975) have suggested that the tightly packed sperm mass of *Sagitta hispida* is enveloped by a thin layer of material, presumably an adhesive mucoid. We did not observe such a layer with TEM or SEM.

It is not known how donorship is determined during mating in *S. schizoptera*. It is not evident whether the actively approaching worm becomes the donor. It is at least certain that a worm which releases its sperm cannot become a donor in the successive mating and that the maturity of the ovary is not related to the mating behavior. Also, sperm transfer is not an essential prelude for egg-laying, because isolated worms lay unfertilized eggs. This means that self-fertilization does not take place under natural conditions.

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